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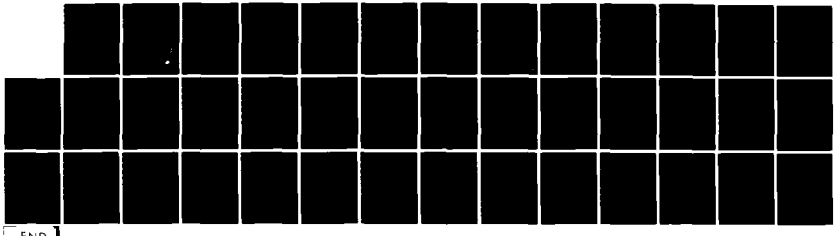

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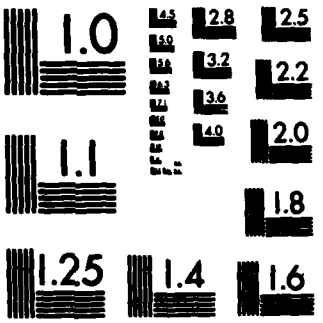

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Report USAFSAM-TR-84-22

GENETIC EFFECTS OF MICROWAVE EXPOSURE ON MAMMALIAN CELLS IN VITRO: VOLUME I

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June 1984

Annual Report for Period February 1980 - June 1981

Prepared for
USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
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NOTICES

This annual report was submitted by the University of Texas Health Science Center, at San Antonio, Texas, under contract F33615-80-C-0607, job order 7757-01-80, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Dr. David N. Erwin (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

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The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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The effects of radiofrequency radiation (RFR) on the DNA repair process in MRC-5 normal human fibroblast cells grown in vitro have been investigated. The power levels chosen, 1 and 10 mW/cm^2, did not result in measurable temperatures above the 37°C incubation temperature at either 350 MHz or 1.2 GHz (continuous or pulse-wave modes). DNA repair was induced by exposure of the cells to ultraviolet light (UV). Repair synthesis was measured by means of a repair replication protocol; i.e., a repair labeling incubation with ^{3}H-thymidine and non-radioactive 5-bromodeoxyuridine (or ^{3}H-BrUdR in early experiments), followed by DNA isolation and two sequential alkali cesium chloride-cesium sulfate density gradient centrifugations. In summary, 1.2-GHz continuous wave (CW) and 350 pulse-wave and continuous-wave RFR, at power levels of 1 and 10 mW/cm^2, did not appear to perturb UV light-induced DNA repair synthesis. A possible stimulation by 350-MHz CW radiation of repair label incorporation during the first hour after UV exposure, at 1 mW/cm^2 (but not at 10 mW/cm^2), remains to be confirmed. No evidence exists of RFR induction, by itself, of DNA damage and repair at 1.2 GHz (CW) and 350-MHz (CW) at a power level of 10 mW/cm^2.

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GENETIC EFFECTS OF MICROWAVE EXPOSURE ON MAMMALIAN CELLS IN VITRO

INTRODUCTION

If mammalian cells are exposed in vitro to temperatures several degrees greater than their normal 37°C growth temperature, the cells will die. Radiofrequency radiation (RFR), at power levels greater than 100 mW/cm^2, can cause measurable temperature increases in biological systems. Any biological effects observed after exposure of mammalian cells to RFR of such power levels could, therefore, be due to heating effects.

An unanswered question has been whether RFR at lower power levels, where measurable heating in the exposure system cannot be detected, causes any transient or permanent alteration in a biochemical or biological endpoint. The DNA molecule--because of its central role in maintaining cell survival, cell function, and genetic inheritance--is a focus for the investigations described in this report.

Two very important cellular processes involving DNA are its replication (synthesis), required for continued cell division, and DNA repair. The latter process allows cells to repair damage induced in their DNA by physical (e.g., ultraviolet light [UV] and X-ray) and chemical hazardous agents (e.g., mutagens and carcinogens). Interference with the process of DNA repair could lead to cell death, cancer, or heritable mutagenic consequences.

The focus of this investigation has been to determine whether exposure of normal human diploid MRC-5 fibroblast cells to RFR--at power levels of 0, 1, and 10 mW/cm^2, and at frequencies of 1.2 GHz and 350 MHz (pulse [PW] or continuous wave [CW] modes)--results in any perturbation of the DNA repair replication process after damage of the DNA by UV light.

RFR EXPOSURE FACILITIES

All RFR exposures were conducted at the USAF School of Aerospace Medicine (USAFSAM), in the anechoic chambers, or in the Narda Model 3801 Transverse Electric Mode (TEM) Transmission Cell. The 1.2-GHz irradiations were performed in the former; a Cober Electronics, Inc., High Power Microwave Generator (Model No. 1831) was employed. The incubation dish, containing the cells to be exposed to RFR, was placed in an especially constructed Plexiglas water bath (to maintain the incubation temperature at 37°C), with the horn directed downward at the dish. A dish with the control cells (non-RFR-exposed) was incubated in a water bath,similar to the one used for the exposed cells in the anechoic chamber; the former was removed from the area of the horn and was surrounded by Eccosorb. Vitek temperature probes were placed into the medium through holes in the covers of the RFR-exposed and control dishes to allow continuous monitoring of the medium temperature during the repair labeling period. In all of these experiments, the temperature in the medium remained at 37°C ± 0.5°C.

The 350-MHz irradiations were performed in a TEM Transmission Cell (NARDA Model No. 8801). A sham cell of similar dimensions was constructed for control incubations. Both cells were fitted with small fans to maximize air circulation; these cells were placed inside a larger anechoic chamber serving as a 37°C warm room. An MCL RF Power Generator (Model no. 15022) was employed for the exposures. Vitek probes were used for temperature monitoring (as described in a previous paragraph). Medium temperatures in the dishes remained at 37°C ± 0.5°C in the warm-room air; water baths were not necessary to maintain the temperature. The PW exposures were performed at 5000 pulses/sec, 10-μs pulse width, with a 0.05 duty factor. For a 1-mW/cm^2 average power level, the peak power was 20 mW/cm^2; for a 10-mW/cm^2 average power level, the peak power was 200 mW/cm^2.

CELL LINE

The MRC-5 normal human diploid fibroblast cell line used in these investigations is an "aging" cell line. It was obtained from the American Type Culture Collection (ATCC), and was kept frozen under liquid nitrogen (in sterile ampoules) until experiments were to be performed. The cells were used only at relatively early passage numbers (before passage 35). Once thawed, the cells were maintained in the biohazard tissue culture laboratories of the Department of Radiology, University of Texas Health Science Center, San Antonio (UTHSCSA), in Basal Minimal Essential Medium (BME) with Hanks' Salts. HEPES at 25 mM was added to maintain the pH in an air atmosphere. The concentration of fetal calf serum was 10%; antibiotics were added.

CELL CULTURE PROCEDURE FOR UV AND RFR EXPOSURE

On the day preceding an RFR exposure, the required numbers of cells in proliferative growth were transported in T-75 flasks to the 37°C incubator at USAFSAM. On the same day, the cells in all of the T-75 flasks were trypsinized to prepare a suspension of single cells. The cells were then distributed in appropriate numbers into large square 24-cm x 24-cm sterile dishes with covers (NUNC; Southland Cryogenics, Carrollton, Tex.) for exposure on the following day. The number of cells seeded resulted in a proliferating cell population (nonconfluent) in the dishes at the time of UV exposure. A Plexiglas circle was used to prevent cells from attaching at an RFR "hot spot" in the center of the dish.

REPAIR REPLICATION PROTOCOL

The standard procedure for repair replication was as follows: One hour before UV irradiation, a portion of the attachment medium was removed from a dish; fresh warm medium, containing 5-fluorodeoxyuridine (FUdR) (to inhibit endogenous thymidine synthesis) and 5-bromodeoxyuridine (5-BrUdR), was added to each dish so that the final concentrations were 1 x 10^{-6}M and 5 x 10^{-6}M, respectively. After 1 hr of incubation at 37°C in the incubator, this prelabeling medium was aspirated; and the attached cells were washed twice with warm phosphate-buffered saline (PBS) to remove UV-absorbing serum proteins. The cells were immediately UV-irradiated in an especially built irradiation chamber

(dose rate 1.4 J/M^2/sec); fresh warm repair-replication-labeling medium was added to the dish immediately after UV exposure. This medium contained 1 x 10^{-6}M FUdR, 5 x 10^{-6}M 5-BrUdR, and 12 µCi/ml ^{3}H-(TdR) (53-59 Ci/mM). Hydroxyurea (HU), at a final concentration of 5 mM, was also added to inhibit incorporation by normal semiconservative DNA synthesis. In early experiments in which the effect of elevated temperature on DNA repair was studied, ^{3}H-BrUdR was used instead of the less expensive and more readily available ^{3}H-TdR. A second dish was similarly UV-irradiated, and labeling medium was added for incubation as in the non-RFR-exposed control. The two dishes were then incubated for either 1, 2, or 3 hr in or outside of the RF field. At the end of this incubation, the labeling medium was aspirated from the dishes, the attached cells were washed with warm BME without serum, and fresh medium with 1 x 10^{-6}M FUdR and 5 x 10^{-6}M 5-BrUdR was added for a final 1-hr incubation in the 37°C incubator. The cells were then washed with cold isotonic salt solution, scraped free into suspension, pelleted in a tube by centrifugation, and quick-frozen by immersion of the tube in ethanol-dry ice.

TEMPERATURE-EFFECT STUDIES

These studies were performed (without RFR exposure) by employing the repair-replication-labeling protocol just described, except that the temperature of incubation during the repair-labeling period immediately following the UV exposure was at 39°C or 42.5°C (or 43°C). This increased temperature was also maintained during the subsequent 1-hr chase incubation. These incubations were performed in a FORMA humidified CO_2 incubator.

DNA ISOLATION PROCEDURE

The pelleted and frozen cells were resuspended in approximately 3 ml of Standard Saline Citrate (SSC): 0.15-M sodium chloride, 0.015-M sodium citrate. The suspension was transferred into a Virtis 5-ml transition flask. After addition of 2% sodium dodecyl sulfate to give a final concentration of 0.1%, the DNA in the suspension was sheared with a microblade at 5000 rpm for 30 sec in ice.

Each lysate was then transferred into a glass 15-ml round bottom-tube, and a sufficient volume of 500-µg/ml RNAse (RNase A, heat-treated, Worthington Biochemical Corp.) was added to give a final concentration of 100 µg/ml. This tube was incubated for 1-hr at 37°C. A sufficient volume of 2.5-mg/ml Pronase (B grade, Cal Biochem, self-digested) was then added to give a final concentration of 500 µg/ml; a 2-hr incubation at 37°C was performed. For deproteinization, an equal volume of chloroform:isoamyl alcohol (24:1) was then added to each tube. The tubes were shaken for 15 min on a reciprocating shaker; they were then centrifuged (in a Beckman PR-J centrifuge) at 3000 rpm for 20 min at 20°C.

The upper aqueous phase was transferred to a new tube, and the chloroform: isoamyl alcohol extraction was repeated. This extraction process was typically repeated 5 times. The final aqueous solution with DNA was transferred into dialysis tubing, and dialyzed overnight at 4°C against 1/10 x SSC (with one change of the 1/10 x SSC).

ALKALI CESIUM CHLORIDE-CESIUM SULFATE DENSITY GRADIENT PROCEDURE

The DNA was subjected to two sequential alkali cesium chloride-cesium sulfate density gradient centrifugations (3) for the separation of normal density repair-replicated DNA from newly synthesized DNA. The procedure is a modification of the technique described by Gautschi et al. (1). For each DNA sample, 4.8 g of CsCl and 1.0 g of Cs_2SO_4 were transferred into a 10-ml beaker, and up to 4.05-ml of aqueous DNA solution were added. The solution was made 0.1 N by the addition of 0.45 ml of 1-N NaOH, and enough 1/10 x SSC to bring the final volume to 5.9 ml. The solutions were then transferred into 13-ml Beckman polypropylene heat-seal tubes, and mineral oil was added to fill the tubes. The centrifugation was performed at 42,000 rpm for 40 hr at room temperature in a Beckman Spinco Type 50 Ti rotor in a Beckman L3-50 (or equivalent) centrifuge.

The alkali gradients were subsequently fractionated into 14 drop fractions (approximately 25 fractions per sample tube) by bottom collection, using an ISCO fraction collector with drop counter. The optical density (O.D.) was continuously monitored at 254 nm with an ISCO UV absorbance monitor (Model UA-5 with a Type 6 optical unit). After fractionation, 20-µl aliquots were transferred onto Whatman 3MM filters so that the incorporated radioactivity could be located. On the basis of the O.D. profile, those fractions containing the bulk parental DNA were combined, added to a volume of preprepared alkali CsCl-Cs_2SO_4 to give a final volume of 5.9 ml, and recentrifuged and fractionated as just described.

Those fractions containing the bulk DNA were again pooled, the pH adjusted to 7.0 with 1-N HCl, and diluted to a volume of 13 ml by addition of deionized glass-distilled water. The DNA was then pelleted out of solution by centrifugation for 20 hr at 42,000 rpm at 10°C in heat-seal tubes in the Beckman centrifuge (Type 50 Ti rotor). The supernatant was carefully aspirated, and the DNA pellet carefully resuspended in 0.5 ml of 1/100 x SSC. Aliquots of 0.1 ml were transferred into 10 x 75 mm siliconized tubes for DNA concentration determination in micrograms per milliliter (µg/ml), and replicate 0.1-ml samples were pipeted into 10 ml of Fisher Scintiverse counting solution for radioactivity determination, in disintegration(s) per minute/milliliter (dpm/ml). The final incorporated repair radioactivity was reported as dpm/µg of DNA.

DNA CONCENTRATION DETERMINATION: THE HINEGARDNER TECHNIQUE

After the 0.1 ml samples (in duplicate) had been dried overnight in the oven at 50°C, a spectrofluorometric technique (2) was used to determine the DNA concentration. This technique was not affected by the fact that the final sample contained denatured DNA. Salmon Testes DNA at different concentrations was used as a concentration standard; 0.1-ml aliquots of these standards were also dried at 50°C. A 0.1-ml volume of diaminobenzoic acid (DABA, Aldrich Chemical Company) solution (0.4-g/ml H_2O) was added to each tube. The uncapped tubes were then placed in a 60°C water bath for 45 min. During this period the purine nucleotides were hydrolyzed, and the exposed sugars reacted with the DABA. After the incubation, 1.0 ml of 1-N HCl was added to the tubes, which were then vortexed to insure thorough mixing. Portions of the samples were transferred, by using Pasteur pipets, into quartz micro cuvettes. The fluorescence of the samples was read on an Aminco-Bowman spectrophotofluorometer at an excitation wavelength of 408 and an emission wavelength of 498. The DNA concentrations, in micrograms of the unknown samples, were determined by comparison to the standard curve.

RESULTS AND DISCUSSION

In the early stages of the project, a series of preliminary experiments were undertaken to establish an appropriate repair labeling protocol for the MRC-5 normal human diploid fibroblasts being used in these investigations. The variables which needed to be considered included:

a) the UV dose to be used to damage the cell DNA and induce its repair;

b) the repair replication labeling time to be used (for the later RFR exposure studies);

c) the shape of the dishes to be used to avoid "hot spots" on the cell attachment surface;

d) the acceptability of the substitution of readily available and inexpensive radiolabeled thymidine (^{3}H-TdR) and nonradioactive BrUdR for the very expensive (and hard to obtain) ^{3}H-BrUdR in the repair labeling protocol; and

e) assurance that elevating the incubation temperature during the repair labeling period, by non-RFR exposure means (water bath and/or incubator temperature settings), would not itself inhibit DNA repair synthesis.

Subsequent to these preliminary studies, experiments were performed to determine whether 1.2-GHz RFR exposure (CW or PW) or 350-MHz RFR exposure (CW or PW), at power levels of 0, 1, or 10 mW/cm^2, had any effect on the UV-induced DNA repair process. The standardized repair replication protocol was employed.

REPAIR LABEL INCORPORATION AS A FUNCTION OF UV DOSE

In this initial study, a 3-hr repair replication period was employed; this was a typical time used in previous repair studies by Dr. Meltz. Proliferating MRC-5 cells were irradiated in round petri dishes at 1.4 J/M^2/sec for different exposure times; the repair incubation temperature was 37°C. The repair label used was ^{3}H-BrUdR. Typical density gradient profiles of first and second alkali gradients are shown in Figures 1 and 2 (10-sec UV); the profiles for the 0-sec UV exposure gradients, in Figures 3 and 4.

The results are summarized in Table 1 and Figure 5. The continuing increase in incorporated repair replication radioactivity with increasing UV dose is apparent out to the 15-sec (21 J/M^2) exposure. A similar experiment was performed later in these investigations, with the MRC-5 cells being UV irradiated in large square 24 cm x 24 cm culture dishes, and with ^{3}H-TdR and nonradioactive BrUdR being used in place of ^{3}H-BrUdR for the repair replication density label. As indicated in Figure 6 and Table 2, a similar increase in incorporated repair radioactivity with increasing UV dose occurred, extending beyond 15-sec (21 J/M^2) of UV exposure.

EDITOR'S NOTE: For the convenience of the reader, all tables have been grouped at the close of this Report.

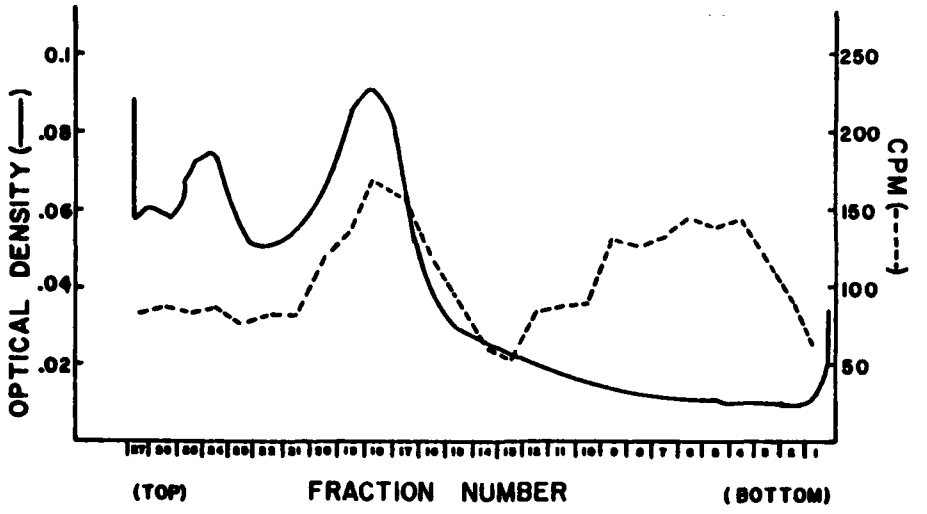

Figure 1. First alkali density gradient profile obtained after 10 sec of UV, using ^{3}H-BrUdR as the repair label (3-hr repair incubation at 37°C).

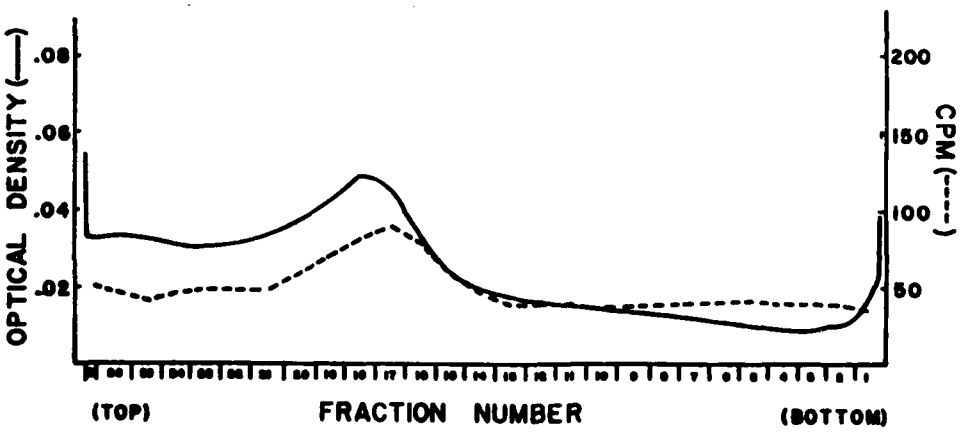

Figure 2. Second alkali density gradient profile obtained after 10 sec of UV, using ^{3}H-BrUdR as the repair label (3-hr repair incubation at 37°C).

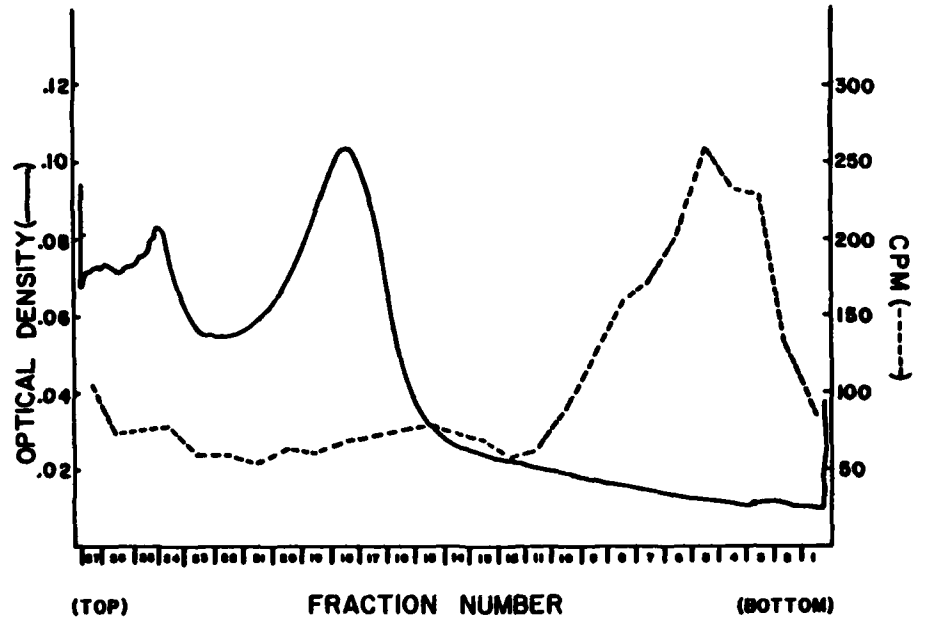

Figure 3. First alkali density gradient profile obtained after 0 sec of UV, using ^{3}H-BrUdR as the repair label (3-hr repair incubation at 37°C).

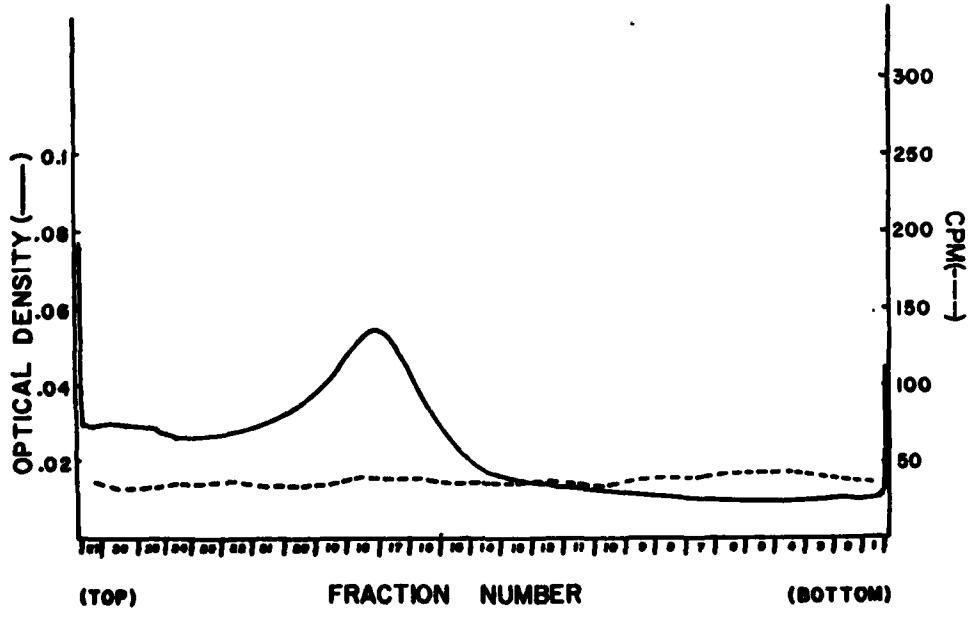

Figure 4. Second alkali density gradient profile obtained after 0 sec of UV, using ^{3}H-BrUdR as the repair label (3-hr repair incubation at 37°C).

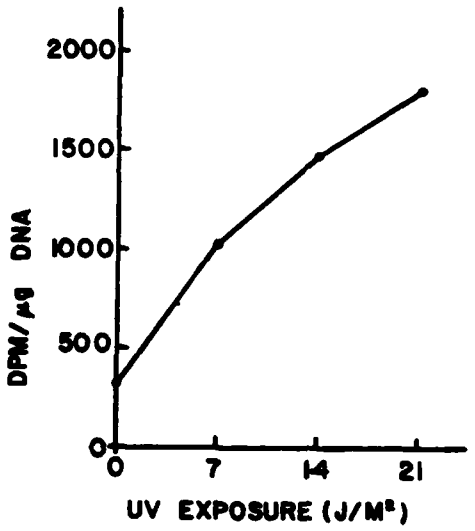

Figure 5. Incorporated repair replication radioactivity with increasing UV dose.

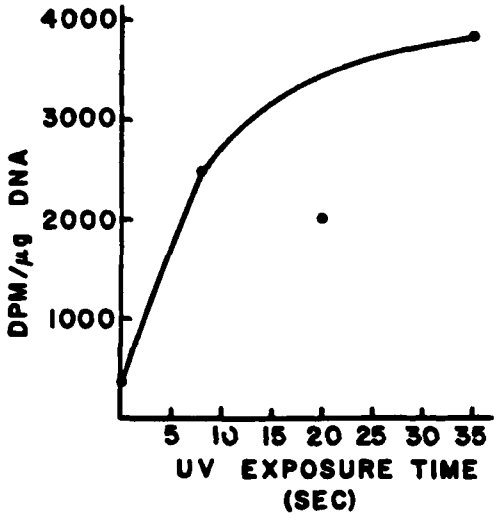

Figure 6. Incorporated repair replication radioactivity with increasing UV exposure time.

RATE OF DNA REPAIR IN UV-IRRADIATED MRC-5 CELLS; EFFECT OF REPAIR LABELING TIME AND OF INCREASED TEMPERATURE

The first of these experiments, which involved a UV dose of 8 sec (11.2 J/M^2), was performed with proliferating MRC-5 cells attached to round petri dishes. The repair label was ^{3}H-BrUdR. The results of these experiments are shown in Table 3 and Figure 7. Prior to 5 hr of repair labeling (at any temperature), the rates of increase of incorporated repair label appear to be similar. At later times, the higher incubation temperature of 39°C may have some effect, and that of 42.5°C has an obvious effect. The decrease in repair label incorporation, observed at 42.5°C, may be related to cell death expected to occur at this temperature and these longer incubation times.

In a later experiment, using ^{3}H-TdR and nonradioactive BrUdR as the repair replication label and exposing the cells to 15 sec of UV (21 J/M^2) in large square culture dishes, the results shown in Table 4 and Figure 8 were obtained. The incubation at 39°C had no effect on the incorporated repair radioactivity as compared to incubation at 37°C during the first 5 hr after UV exposure.

CHANGE IN REPAIR LABEL

The change in the radioactive label for measuring DNA repair was already mentioned. In ^{3}H-BrUdR experiments, the specific activity of the ^{3}H-BrUdR was 10 µCi/ml; the final BrUdR molarity, 5×10^{-6}M. In the ^{3}H-TdR experiments, the ^{3}H-TdR specific activity was 12 µCi/ml; the final BrUdR molarity, again, 5×10^{-6}M. The final molar ratio of BrUdR:TdR was 20:1; this was selected after an examination of values previously described in the literature. In Figures 9 and 10 are shown first and second alkali density gradient profiles obtained after 8 sec of UV, using ^{3}H-TdR as the repair label (3-hr repair incubation at 37°C). These profiles, which can be compared to the ^{3}H-BrUdR profiles in Figures 1 and 2 (already described), indicate a satisfactory density difference: between pre-existing DNA, which has incorporated label by repair replication--and newly synthesized DNA, of greater density because of more extensive BrUdR incorporation.

INVESTIGATION OF THE RFR EFFECT OF 1.2 GHZ AND 350 MHZ ON UV-INDUCED DNA REPAIR

This series of investigations was performed to determine whether RFR exposure at power levels of 0, 1, or 10 mW/cm^2 would result in a measurable alteration in the rate of DNA repair synthesis induced by UV irradiation. After thymine dimers are produced in the DNA by UV light exposure, cellular enzymes nick the DNA (break the DNA single strand) alongside the dimer, excise a region of DNA approximately 100 bases long containing the dimer, and repair-synthesize the DNA which has been excised. A subsequent step is the rejoining of the break between repair-synthesized and adjoining old DNA. When RFR exposure of MRC-5 cells occurs during the repair period, several molecular level steps can be interfered with if the DNA molecule is perturbed by the RFR. Any change in the rate of incorporation of repair label into DNA, when measured by the repair replication protocol employed in these investigations, would indicate an effect on at least one of the repair steps up to, but not including, the final rejoining step.

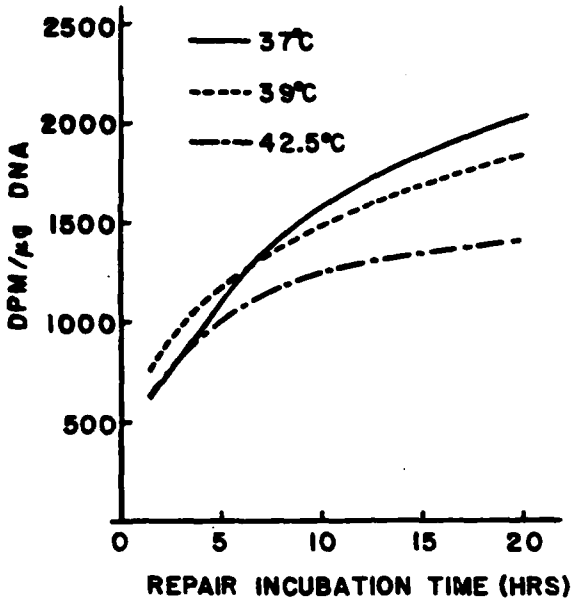

Figure 7. Rate of DNA repair at different temperatures after 8 sec of UV using ^{3}H-BrUdR as the repair label. Proliferating MRC cells were attached to round petri dishes.

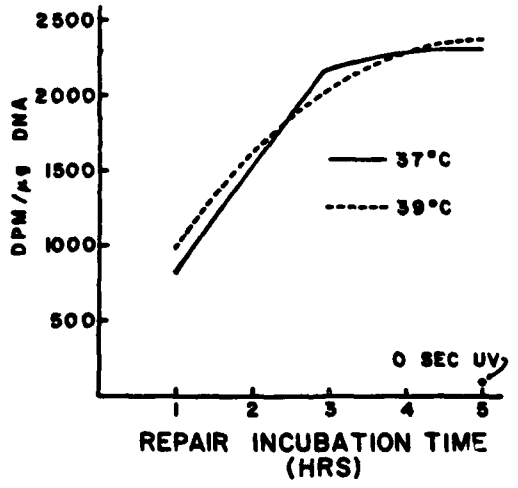

Figure 8. Rate of DNA repair at different temperatures after 15 sec of UV using ^{3}H-TdR (and nonradioactive BrUdR) as the repair label. Proliferating MRC-5 cells were attached to large square culture dishes.

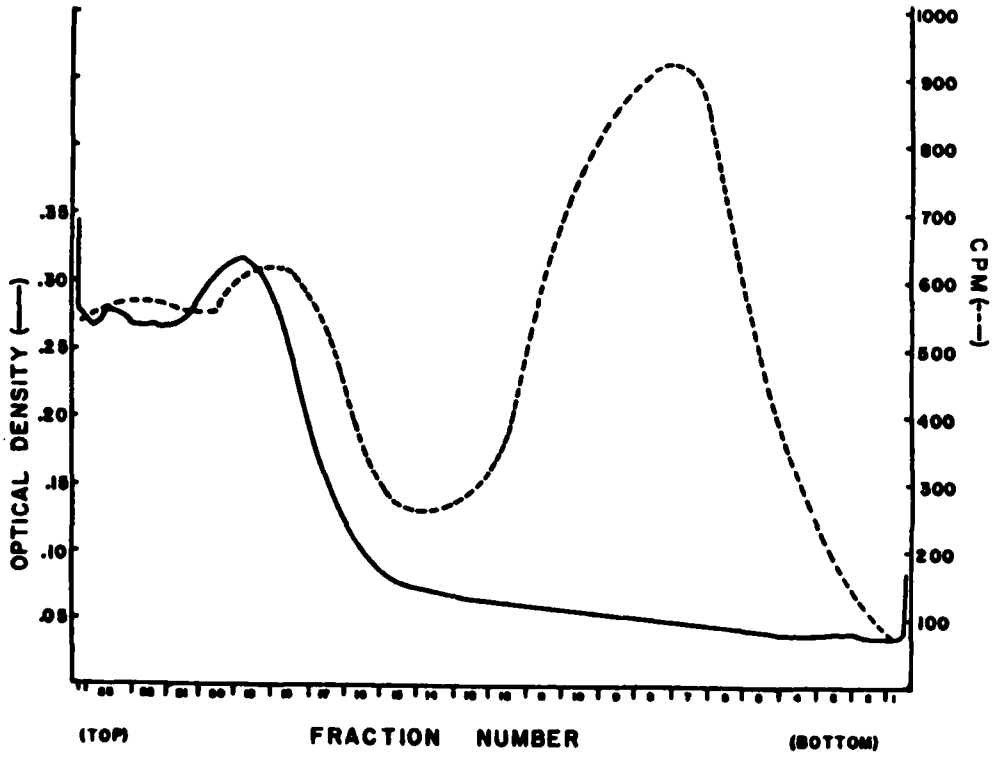

Figure 9. First alkali density gradient profile obtained after 8 sec of UV, using ^{3}H-TdR as the repair label (3-hr repair incubation at 37°C).

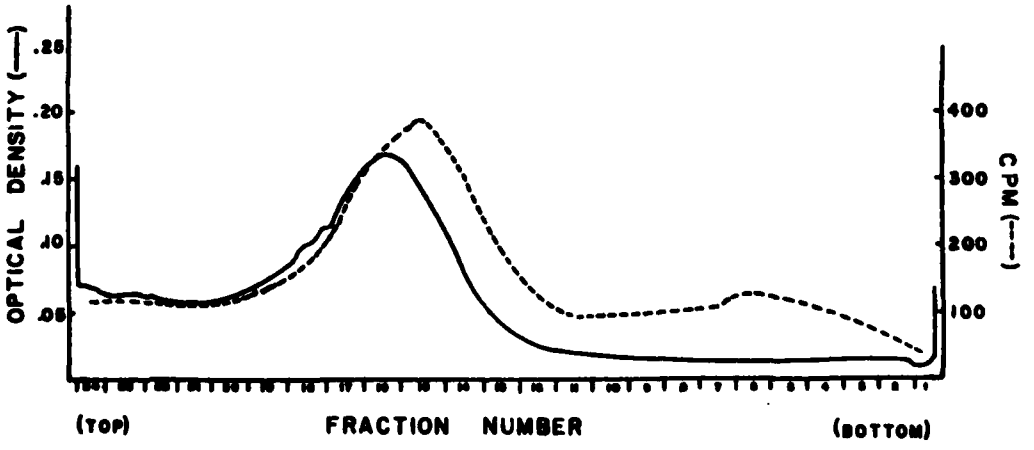

Figure 10. Second alkali density gradient profile obtained after 8 sec of UV, using ^{3}H-TdR as the repair label (3-hr repair incubation at 37°C).

The results of our investigations, upon RFR exposure of UV-exposed cells for 3 hr at a constantly monitored medium temperature of 37°C, are given in Table 5 for 1.2-GHz CW exposure; Table 6, for 1.2-GHz PW exposure; Table 7, for 350-MHz CW exposure; and Table 8, for 350-MHz PW exposure. In the following discussion, we examine comparative data from the different experiments. Subsequent to obtaining these data we became aware that the operating settings for the 1.2-GHz PW exposure might not have provided the PW exposure desired. The 1.2-GHz exposures will, therefore, be repeated in the second year of the project (4).

CONTROL STUDIES

No UV, No RFR exposure, Generator on

For each frequency and mode, a background incorporated radioactivity experiment was performed, with cells incubated in dishes in their RFR exposure position or in their control position. For the 1.2-GHz exposure sets, the dishes in the anechoic chamber were either under the horn (RFR exposure position), or away from the horn (control position) and surrounded by Eccosorb. The RF generator was on. For the 350-MHz exposure sets, the dishes were either in the TEM or in the separate sham cell; the generator connected to the TEM was on.

The data in Table 9 show similar background values for the two positions in each experimental set, with the exception of the 350-MHz PW set. Our check of the Hinegardner procedure data for the DNA concentration determination, and of the counts per minute (cpm) data and counting efficiency values for the disintegration(s) per minute (dpm) determination, showed that the replicates in each analysis were very close, and within the normal ranges. We therefore have no explanation for the high value (594 dpm/μg) observed.

Does RFR Exposure of MRC-5 Cells Induce DNA Repair?

This question was investigated for each frequency and mode. The data, (Table 10) show comparable radioactivity values with and without RFR exposure at 1.2 GHz and at 350-MHz CW. A high incorporated radioactivity value was obtained with RFR exposure for 350 MHz PW, but the DNA control without RFR was not recovered for analysis. Since the background value (Table 9) was so relatively high, no conclusion could be drawn as to whether 350-MHz PW did or did not induce DNA repair.

Comparison of the Rate of UV-Induced Repair in the RFR Exposure Position Vs. the Control Position, With the Generator On, But Without RFR Exposure

The data for the different frequencies and modes are presented in Table 11. No real difference exists when the cells are incubated for UV-induced repair label incorporation in either the exposure or control position.

Also apparent is the fact that experiment-to-experiment variability occurs in the absolute values of the disintegrations per minute per microgram of the repair radioactivity, incorporated after UV irradiation, for both the 1-hr and

3-hr labeling periods. This finding is of concern, but it is a reality previously encountered in experimental DNA repair studies by Dr. Meltz. Each experimental set is internally controlled with similar cell populations, similar growth medium and, especially, the same labeling medium, and shows a consistency which assures us of the integrity of each experiment.

Does RFR Affect UV-induced DNA Repair?

The data for each frequency and mode are presented in Table 12.

Some variability is present in the 1-mW/cm^2 data; and a possibility exists (suggested by only one data point) that a 1-hour incubation in a 350-MHz PW field at 1 mW/cm^2 might increase the initial rate of DNA repair (1940 dpm/µg) with RFR exposure vs. 940 dpm/µg without RFR exposure). Nevertheless, the values for UV-induced repair incorporated radioactivity occurring in RFR fields at 1.2 GHz and 350 MHz at 10 mW/cm^2 are consistently similar to their controls. The 350-MHz PW result at 1 mW/cm^2 remains to be confirmed; the remaining data to this point do not suggest any RFR effect, at a power level up to 10 mW/cm^2, on the DNA repair process.

As an additional check on the internal consistency of our experiments, selected data already presented were retabulated.

In Table 13 are shown the 3-hr incubation data from a given experimental set, with or without RFR exposure; also shown are the 3-hr data from the same set for the UV-induced repair in the RFR exposure position from one of the earlier described control experiments (i.e., the generator on, exposure position vs. control position experiment) which was performed on a different day. As shown clearly in Table 13, the variability in the absolute magnitudes obtained in the RFR exposure experiments reflects very closely that in the control UV studies between the sets. This finding further supports the statement of internal consistency made in the foregoing paragraph.

CONCLUSIONS

In summary, 1.2-GHz CW and 350-MHz PW and CW RFR, at power levels of 1 and 10 mW/cm^2, do not appear to perturb UV-light-induced DNA repair synthesis in a normal human cell line maintained in vitro. Yet to be confirmed remains a possible stimulation by 350-MHz CW radiation of repair label incorporation during the first hour after UV exposure, at 1 mW/cm^2 (but not at 10 mW/cm^2). No evidence has been found for RFR induction, by itself, of DNA damage and repair at 1.2 GHz (CW) and 350 MHz (CW) at a power level of 10 mW/cm^2.

REFERENCES

1. Gautschi,J. R., et al., "Evidence for DNA repair replication in unirradiated mammalian cells--Is it an artifact?" Biochim Biophys Acta, Vol. 281, pp. 324-328, June 1972.

2. Hinegardner, R. T., "An improved fluorometric assay for DNA." Analytical Biochem, Vol. 39, pp. 197-201, Jan 1971.

REFERENCES

3. Meltz, M. L., "DNA repair in baboon glucolar macrophages: A system for assessing biohazards on materials." Environ Res, Vol. 11, pp. 359-366, Oct 1976.

4. Meltz, M.L., Walker, K.A., "Genetic Effects of Microwave Exposure on Mammalian Cells In Vitro: Volume II." USAFSAM-TR-84-24. (In Press)

TABLES 1 - 13

EDITOR'S NOTE: Throughout the series of tables in this Report, the following abbreviations are frequently used--

B/A ratio = channels ratio, liquid scintillation counter
Bkg = background
dpm = disintegration(s) per minute
%Eff = percent efficiency

TABLE 1. INCORPORATION OF REPAIR REPLICATION RADIOACTIVITY IN HUMAN MRC-5 CELL DNA DURING A 3-HR LABELING PERIOD AT 37°C AFTER DIFFERENT UV LIGHT EXPOSURES

[^{3}H-BrUdR Label and Round Petri Dishes]

UV dose (J/M^2)	Total DNA recovered (µg)	dpm/µg DNA
0	0.75	326
7	1.85	1039
14	0.6	1488
21	0.5	1800

TABLE 2. INCORPORATION OF REPAIR REPLICATION RADIOACTIVITY IN HUMAN MRC-5 CELL DNA DURING A 3-HR LABELING PERIOD AT 37°C AFTER DIFFERENT UV LIGHT EXPOSURE TIMES (1.4 J/M^2/SEC)

[^{3}H-TdR Label and Square Culture Dishes]

UV exposure (sec)	Incubation time (hr)	cpm [a] / .1 ml	Average [b] -Bkg.	dpm [c] / .1 ml	µg DNA [d] / .1 ml	dpm/µg DNA
0	3	713 748	707	1754	4.75	369
8	3	2468 2483	2452	6239	2.5	2496
20	3	1894 1717	1782	5843	2.9	2015
35	3	651 614	609	1542	0.4	3855

[a] *Values given are for duplicate samples taken after final DNA pelleting and counted in Scintiverse.*

[b] *Background value is 23 cpm.*

[c] *DPM value obtained by correcting cpm for percent efficiency from quench curve.*

[d] *Value given is the average of duplicate samples taken after final DNA pelleting and assayed by the method of Hinegardner.*

TABLE 3. RATE OF DNA REPAIR IN HUMAN MRC-5 CELLS REPAIR LABELED AT DIFFERENT TEMPERATURES AFTER AN 8-SEC UV LIGHT EXPOSURE

Temp	Incubation time (hr)	cpm [a] / .1 ml	Average [b] -Bkg.	dpm [c] / .1 ml	µg DNA [d] / .1 ml	dpm/µg DNA
37°C						
(Control- No. UV)	5	41 37	11	20	1.17	17
	1.5	444 440	414	1056	1.7	621
	3	740 785	735	1997	2.2	908
	5	860 844	824	2180	2.25	969
	8	826 900	835	2239	1.58	1417
	20	963 953	930	2460	1.2	2050
39°C						
(Control- No. UV)	5	58 55	25 *	49	1.78	28
	1.5	609 664	609	1628	2.15	757
	3	943 688	788	2090	2.18	959
	5	774 868	793	2076	1.6	1298
	8	762 696	697 *	1815	1.35	1344
	20	933 917	893 *	2362	1.29	1831

(Cont'd. on next page)

For key to letters a, b, c, and d, and asterisk, refer to footnotes on next page.

TABLE 3 (CONT'D.)

Temp	Incubation time (hr)	cpm [a] .1 m	Average [b] -Bkg.	dpm [c] .1 ml	µg DNA [d] .1 ml	dpm/µg DNA
42.5°C						
(Control- No. UV)	5	54 61	26*	50	1.32	38
	1.5	498 492	463*	1206	1.93	625
	3	935 845	858*	2270	2.7	841
	5	747 723	703*	1860	1.85	1005
	8	685 669	645*	1671	1.44	1160
	20	1072 1051	1030*	2725	1.93	1412

[a] *Values given are for duplicate samples taken after final DNA pelleting and counted in Scintiverse.*

[b] *Background value for * samples is 32; and, for others, 28.*

[c] *DPM value obtained by correcting cpm for percent efficiency from quench curve.*

[d] *Value given is the average of duplicate samples taken after final DNA pelleting and assayed by the method of Hinegardner.*

TABLE 4. EFFECT OF TEMPERATURE ON INCORPORATION OF REPAIR REPLICATION RADIOACTIVITY IN HUMAN MRC-5 CELLS DURING A 3-HR LABELING PERIOD AFTER A 15-SEC UV EXPOSURE

[^{3}H-TdR Label and Square Culture Dishes]

Temp	UV exposure (sec)	Incubation time (hr)	cpm / .1 ml [a]	Average [b] -Bkg.	dpm [c] / .1 ml	µg DNA [d] / .1 ml	dpm/µg DNA
37°C	0	5	91 83	60	149	1.45	103
37°C	15	1	534 565	523	1473	1.8	818
37°C	15	3	340 361	319	881	0.4	2203
37°C	15	5	1280 1228	1227	3718	1.6	2324
39°C	0	5	61 57	32	71	0.6	118
39°C	15	1	359 334	320	894	0.9	993
39°C	15	3	814 815	788	2251	1.1	2046
39°C	15	5	98 102	73	190	0.08	2375

[a] *Values given are for duplicate samples taken after final DNA pelleting and counted in Scintiverse.*

[b] *Background value is 27 cpm.*

[c] *DPM value obtained by correcting cpm for percent efficiency from quench curve.*

[d] *Value given is the average of duplicate samples taken after final DNA pelleting and assayed by the method of Kinegardner.*

TABLE 5. DNA REPAIR STUDY: 1.2-GHz CONTINUOUS-WAVE RADIATION

[Table 1 in Volume II[a]]

	Incubation Time (hr)	cpm / .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm / .1 ml	µg DNA / .1 ml	dpm / µg DNA
A. UV-induced repair replication in anechoic chamber with generator on, in Exposure vs. Control Position. The cells were not exposed to RFR.										
1. Control Position	1	377 280	329	305	.381 .379	.380	36.5	836	1.18	708
2. Exposure Position	1	191 201	196	172	.400 .405	.403	38.0	453	0.64	708
3. Control Position	3	506 496	501	477	.369 .381	.375	36.0	1,325	0.96	1,380
4. Exposure Position	3	284 391	338	314	.399 .374	.387	37.0	849	0.63	1,348
B. Background level of radioactivity incorporated into DNA of cells without UV exposure in anechoic chamber with generator on, in Exposure vs. Control Position. The cells were not exposed to RFR.										
1. Control Position	3	117 143	130	98	.410 .401	.406	40.0	245	0.94	261
2. Exposure Position	3	84 79	82	50	.457 .435	.446	42.5	118	0.56	211

(Cont'd. on next page)

[a] *Refer to item 4 in the preceding list of "References."*

TABLE 5 (CONT'D.)

	Incubation time (hr)	cpm .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm .1 ml	µg DNA .1 ml	dpm µg DNA
C. Assay for induction of repair synthesis by 1.2-GHz continuous-wave radiation in non-UV irradiated cells.										
1. Control	3	151 149	150	118	.413 .405	.409	40.0	295	0.70	421
2. RFR (10 mW/cm^2)	3	176 170	173	141	.400 .378	.389	38.7	364	0.82	444
D. Effect of 1.2-GHz continuous-wave radiation at 1 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	42 42	42	10	.500 .536	.518	45.2	22	0.02[b]	1,100
2. 1 mW/cm^2	1	222 227	225	193	.384 .400	.392	39.0	495	0.61	811
3. Control	2	107 114	111	79	.429 .403	.416	40.5	195	0.92	212[c]
4. 1 mW/cm^2	2	240 207	224	192	.376 .407	.392	39.0	492	0.45	1,093
5. Control	3	297 229	298	266	.390 .379	.385	38.5	691	0.54	1,280
6. 1 mW/cm^2	3	194 177	186	154	.388 .414	.401	39.5	390	0.29[b]	1,345

(Cont'd. on next page)

[b] *Low DNA yield*

[c] *Value not acceptable; too low for UV-exposed cells.*

TABLE 5 (CONT'D.)

	Incubation time (hr)	cpm .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm .1 ml	µg DNA .1 ml	dpm µg DNA
E. Effect of 1.2-GHz continuous-wave radiation at 10 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	302 195	249	225	.387 .400	.394	37.5	600	1.34	448
2. 10 mW/cm^2	1	141 160	151	127	.407 .425	.416	39.0	326	0.64	509
3. Control	2	291 396	344	320	.396 .374	.385	37.0	865	0.86	1,006
4. 10 mW/cm^2	2	304 289	297	273	.376 .363	.370	35.8	763	0.8	954
5. Control	3	238 205	222	198	.375 .404	.390	37.2	532	0.58	917
6. 10 mW/cm^2	3	70 80	75	51	.471 .437	.454	41.2	124	0.1[b]	1,240

TABLE 6. DNA REPAIR STUDY: 1.2-GHz PULSE-WAVE RADIATION

[Table 2 in Volume II[a]]

	Incubation time (hr)	cpm .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm .1 ml	µg DNA .1 ml	dpm µg DNA
A. UV-induced repair replication in anechoic chamber with generator on, in Exposure vs. Control Position. The cells were not exposed to RFR.										
1. Control Position	1	94 107	101	77	.376 .424	.400	39	197	0.7	281
2. Exposure Position	1	69 69	69	45	.485 .478	.482	44	102	0.3	340
3. Control Position	3	120 118	119	95	.425 .423	.424	41	232	0.4	580
4. Exposure Position	3	139 138	139	115	.402 .398	.400	39	295	0.51	578
B. Background level of radioactivity incorporated into DNA of cells without UV exposure in anechoic chamber with generator on, in Exposure vs. Control Position. The cells were not exposed to RFR.										
1. Control Position	3	37 40	39	15	.555 .589	.572	47	32	0.4	80
2. Exposure Position	3	43 40	42	18	.547 .525	.536	45	40	0.45	89

(Cont'd. on next page)

[a] *Refer to item 4 in the preceding list of "References."*

TABLE 6 (CONT'D.)

	Incubation time (hr)	cpm / .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm / .1 ml	µg DNA / .1 ml	dpm / µg DNA
C. Assay for induction of repair synthesis by 1.2-GHz pulse-wave radiation in non-UV irradiated cells.										
1. Control	3	51 53	52	28	.480 .509	.495	43	65	0.95	68
2. RFR (10 mW/cm^2)	3	33 32	33	9	.545 .593	.569	47	19	0.4	48
D. Effect of 1.2-GHz pulse-wave radiation at 1 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	57 57	57	33	.491 .446	.469	43	77	0.2	385
2. 1 mW/cm^2	1	73 73	73	49	.438 .424	.431	41	120	0.28	429
3. Control	2	97 93	95	71	.453 .467	.460	43	165	0.26	635
4. 1 mW/cm^2	2	126 132	129	105	.396 .416	.406	40	263	0.42	626
5. Control	3	64 57	61	37	.492 .456	.474	44	84	0.05	1,680
6. 1 mW/cm^2	3	167 149	158	134	.389 .422	.406	40	335	0.45	744

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TABLE 6 (CONT'D.)

	Incubation time (hr)	cpm / .1 mT	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm / .1 mT	µg DNA / .1 mT	dpm / µg DNA
E. Effect of 1.2-GHz pulse-wave radiation at 10 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	81 81	81	57	.450 .456	.453	40	143	0.5	286
2. 10 mW/cm^2	1	38 43	41	17	.540 .604	.572	47	36	0.15	240
3. Control	2	85 85	85	61	.452 .470	.461	41	149	0.35	426
4. 10 mW/cm^2	2	119 114	117	93	.386 .424	.405	38	245	0.33	742
5. Control	3	105 107	106	82	.419 .452	.436	39	210	0.38	553
6. 10 mW/cm^2	3	146 151	149	125	.413 .400	.407	38	329	0.6	548

TABLE 7. DNA REPAIR STUDY: 350-MHz CONTINUOUS-WAVE RADIATION

[Table 3 in Volume II[a]]

	Incubation time (hr)	cpm / .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm / .1 ml	µg DNA / .1 ml	dpm / µg DNA
A. UV-induced repair replication in TEM Chamber with generator on, vs. in Sham Chamber. The cells were not exposed to RFR.										
1. Sham Chamber	3	385 469	427	402	.309 .321	.315	32	1,256	2.1	598
2. Generator on, TEM Chamber	3	311 306	309	284	.327 .333	.330	33	861	1.35	638
B. Background level of radioactivity incorporated into DNA of cells without UV exposure in TEM Chamber with generator on, vs. in Sham Chamber. The cells were not exposed to RFR.										
1. Sham Chamber	3	57 57	57	33	.491 .491	.491	43	77	1.3	59
2. Generator on, TEM Chamber	3	48 45	47	22	.416 .477	.447	40	55	0.63	87

(Cont'd. on next page)

[a] *Refer to item 4 in the preceding list of "References."*

TABLE 7 (CONT'D.)

	Incubation time (hr)	cpm .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm .1 ml	µg DNA .1 ml	dpm µg DNA
C. Assay for induction of repair synthesis by 350-MHz continuous-wave radiation in non-UV irradiated cells.										
1. Control	3	42 46	44	19	.476 .422	.449	40	48	0.63	76
2. RFR (10 mW/cm^2)	3	55 51	53	28	.454 .400	.427	39	72	1.0	72
D. Effect of 350-MHz continuous-wave radiation at 1 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	155 146	151	126	.367 .372	.370	36	350	0.8	438
2. 1 mW/cm^{2}on	1	116 119	118	93	.387 .372	.380	36	258	0.62	417
3. Control	2	183 167	175	150	.362 .359	.361	35	429	0.68	631
4. 1 mW/cm^2	2	117 118	118	93	.370 .364	.367	35	266	0.41	649
5. Control	3	135 135	135	110	.377 .370	.374	36	306	0.38	805
6. 1 mW/cm^2	3	210 210	210	185	.352 .347	.350	35	529	0.69	767

(Cont'd. on next page)

TABLE 7 (CONT'D.)

	Incubation time (hr)	cpm .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm .1 ml	µg DNA .1 ml	dpm µg DNA
E. Effect of 350-MHz continuous-wave radiation at 10 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	89 91	90	66	.443 .422	.433	40	165	0.78	212
2. 10 mW/cm^2	1	118 123	121	97	.389 .389	.389	37	262	1.1	238
3. Control	2	128 118	123	99	.433 .358	.396	38	261	0.85	307
4. 10 mW/cm^2	2	119 120	120	96	.403 .420	.412	39	246	0.7	351
5. Control	3	77 80	79	55	.467 .417	.442	40	138	0.42	329
6. 10 mW/cm^2	3	170 165	168	144	.382 .378	.380	37	389	0.95	409

TABLE 8. DNA REPAIR STUDY: 350-MHz PULSE-WAVE RADIATION

[Table 4 in Volume II[a]]

	Incubation time (hr)	cpm .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm .1 ml	µg DNA .1 ml	dpm µg DNA
A. UV-induced repair replication in TEM Chamber with generator on, vs. in Sham Chamber. The cells were not exposed to RFR.										
1. Sham Chamber	1	878 862	870	847	.386 .380	.383	37	2,289	1.7	1,347
2. Generator on, TEM Chamber	1	632 653	643	620	.386 .372	.379	37	1,676	1.25	1,341
3. Sham Chamber	3	1,331 1,356	1,344	1,320	.368 .371	.370	36	3,667	1.78	2,060
4. Generator on, TEM Chamber	3	1,278 1,269	1,274	1,250	.374 .379	.377	36	3,472	1.45	2,395
B. Background level of radioactivity incorporated into DNA of cells without UV exposure in TEM Chamber with generator on, vs. in Sham Chamber. The cells were not exposed to RFR.										
1. Sham Chamber	3	356 357	357	334	.433 .435	.434	38	879	1.48	594
2. Generator on, TEM Chamber	3	154 150	152	129	.409 .402	.406	37	349	1.24	281

(Cont'd. on next page)

[a] *Refer to item 4 in the preceding list of "References."*

TABLE 8 (CONT'D.)

	Incubation time (hr)	cpm .1 ml	Avg.	-Bkg	B/A Ratio	Avg.	%Eff	dpm .1 ml	µg DNA .1 ml	dpm µg DNA
C. Assay for induction of repair synthesis by 350-MHz pulse-wave radiation in non-UV irradiated cells.										
1. Control	3	54 56	55	32	.500 .490	.495	42	76	0	---
2. RFR (10 mW/cm^2)	3	239 239	239	216	.424 .447	.436	39	554	0.8	692
D. Effect of 350-MHz pulse-wave radiation at 1 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	121 112	117	94	.475 .446	.461	40	235	0.25	940
2. 1 mW/cm^2	1	535 543	539	516	.433 .421	.427	38	1,358	0.7	1,940
3. Control	2	631 620	626	603	.428 .418	.423	38	1,587	0.75	2,116
4. 1 mW/cm^2	2	712 722	717	694	.428 .421	.425	38	1,826	0.75	2,435
5. Control	3	350 347	349	326	.417 .426	.422	38	858	0.5	1,716
6. 1 mW/cm^2	3	333 523	428	405	.349 .340	.345	33	1,227	0.7	1,753

(Cont'd. on next page)

TABLE 8 (CONT'D.)

	Incubation time (hr)	cpm / .1 ml	Avg.	-Bkg	B/A ratio	Avg.	%Eff	dpm / .1 ml	µg DNA / .1 ml	dpm / µg DNA
E. Effect of 350-MHz pulse-wave radiation at 10 mW/cm^2 on UV-induced DNA repair.										
1. Control	1	483 457	470	447	.376 .396	.386	37	1,208	1.24	974
2. 10 mW/cm^2	1	567 570	569	545	.382 .377	.380	37	1,473	1.58	932
3. Control	2	693 754	724	700	.372 .359	.366	36	1,944	1.2	1,620
4. 10 mW/cm^2 [a]	2	535 523	529	506	.376 .372	.374	36	1,406	0.98	1,434
5. Control	3	910 922	916	893	.365 .369	.367	36	2,481	1.48	1,676
6. 10 mW/cm^2	3	910 893	902	878	.369 .367	.368	36	2,439	1.43	1,706

[a] *These data are from a continuous-wave exposure, not a pulse-wave exposure.*

TABLE 9. BACKGROUND INCORPORATED RADIOACTIVITY IN BULK (O.D. PEAK) DNA

[No UV; No RFR Exposure; Generator On]

	dpm/μg (3-hr labeling incubation)	
Frequency/Mode	RFR exposure position	Control position
1.2-GHz CW	211	261
1.2-GHz PW [a]	89	80
350-MHz CW	87	59
350-MHz PW	281	594 [b]

[a] *Not likely to be a true pulse-wave (PW) exposure.*

[b] *A review of Hinegardner DNA concentration assay data, cpm data, and counting efficiency values showed replicate values were all very close, and/or within the normal ranges. No explanation for high value.*

TABLE 10. DOES RFR EXPOSURE INDUCE DNA REPAIR?

[No UV, With and Without 10 mW/cm^2 RFR Exposure for 3 Hr]

Frequency/Mode	dpm/µg	
	With RFR	Without RFR
1.2-GHz CW	444	421
1.2-GHz PW [a]	48	68
350-MHz CW	72	76
350-MHz PW	692	No DNA Recovered

[a] *Not likely to be a true pulse-wave (PW) exposure.*

TABLE 11. COMPARISON OF UV-INDUCED DNA REPAIR IN EXPERIMENTAL SETS PERFORMED AT DIFFERENT TIMES

[UV Irradiated; Generator On; But No RFR Exposure]

Frequency/Mode	dpm/µg			
	RFR exposure position		Control position	
	1 Hr	3 Hr	1 Hr	3 Hr
1.2-GHz CW	708	1,348	708	1,380
1.2-GHz PW [a]	340	578	281	580
350-MHz CW		638		598
350-MHz PW	1,341	2,395	1,347	2,060

[a] *Not likely to be a true pulse-wave (PW) exposure.*

TABLE 12. DNA REPAIR DURING RFR EXPOSURE AFTER UV IRRADIATION

Frequency/Mode	Repair incubation time (hr)	dpm/µg			
		1 mW/cm^2		10 mW/cm^2	
		+RFR	Control	+RFR	Control
1.2-GHz CW	1	811	1,100 [a]	509	448
	2	1,093	212 [b]	954	1,006
	3	1,345 [a]	1,280	1,240	917
1.2-GHz PW [c]	1	429	385	240	286
	2	626	635	742	426
	3	744	1,680 [a]	548	553
350-MHz CW	1	417	438	238	212
	2	649	631	351	307
	3	767	805	409	329
350-MHz PW	1	1,940	940	932	974
	2	2,435	2,116	(Received CW)	1,620
	3	1,753	1,716	1,706	1,676

[a] *Extremely low DNA value, error likely in dpm/µg.*

[b] *Unacceptably low value for UV-irradiated cells*

[c] *Not likely to be true pulse-wave (PW) exposure.*

TABLE 13. COMPARISON OF 3-HR INCORPORATED REPAIR RADIOACTIVITY AFTER UV IRRADIATION; RFR EXPOSURE AND CONTROL VALUES VS. CONTROL (NO RFR) IN EXPOSURE POSITION PERFORMED ON A DIFFERENT DAY

Frequency/Mode	dpm/µg				
	Control experiment	RFR effect on DNA repair experiment			
	RFR position	1 mW/cm^2		10 mW/cm^2	
		+RFR	Control	+RFR	Control
1.2-GHz CW	1,348	1,345	1,280	1,240	917
1.2-GHz PW [a]	578	744	[b]	548	553
350-MHz CW	638	767	805	409 [c]	329 [c]
350-MHz PW	2,395	1,753	1,716	1,706	1676

[a] *Not likely to be a true pulse-wave (PW) exposure.*

[b] *Low DNA yield*

[c] *The 10 mW/cm^2 values for the 350 MHz continuous-wave (CW) exposure are lower than expected.*

END

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